

The Karyotype of the Treefrog *Rhacophorus reinwardtii* (Boie) from Xishuangbanna Daizu Autonomous Prefecture, Yunnan Province, China

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Abstract. -The Chinese Flying Treefrog, *Rhacophorus reinwardtii*, has a diploid chromosome number of 26, with 5 pairs of large and 8 pairs of small chromosomes. A secondary constriction occurs in the paracentric region of the short arm of the first chromosome pair. Submetacentric chromosomes occur on large pairs 2, 3, and 4. No other rhacophorid frogs are known to have these karyotypic features. The significance of these findings for chromosomal evolution in rhacophorid frogs is considered.

Key words: Anura, Rhacophoridae, *Rhacophorus*, Cytotaxonomy, Yunnan, China.

Introduction

Chinese rhacophorid frogs are numerous (about 40 species) and their taxonomic position, especially their supraspecific classification, is controversial (Liu and Hu 1961, Liem 1970, Jiang et al. 1987). In recent years we have been attempting to use cytotaxonomical methods and data to clarify the situation.

Karyotypes have been reported for only a few of the many species of Chinese rhacophorid frogs. Gao et al. (1985) reported the karyotype and its C-banding of *Rhacophorus dennysi*. Yang and Wu (1986) reported the karyotype of a new tree frog, *Rhacophorus gongshanensis*. Tan et al. 1987a, reported the karyotype, C-banding and Ag-NORs of *Rhacophorus chenfui*. Tan (1987) reported an extraordinary condition in which different diploid numbers ($2n=26$ and $2n=16$) were found in single individuals of the small treefrog, *Philautus doriae*. This paper reports the karyotype of *Rhacophorus reinwardtii*.

Methods

In a survey of the herpetofauna of Xishuangbanna Daizu Autonomous Prefecture, Yunnan Province, China, during May and June, 1986, over 50 specimens of the Chinese Flying Treefrog,

Rhacophorus reinwardtii, were collected in the vicinity of Mengleng, at Buyuan River, 19 km WNW of Mengxing (21° 55' N 101° 22' E). Fifteen of these specimens were used to examine its karyotype. The reproductive habits of this treefrog were observed and described in another paper (Tan et al. 1987b).

Mitotic chromosomes were prepared from bone marrow tissues using a modified direct chromosome-making method. An injection of 0.2 ml of a 0.1% solution of colchicine was injected intraperitoneally, and after 10 hours the specimens were sacrificed. The long bones were dissected from the four limbs. The two ends of the bones were removed and the bone marrow cells were washed directly onto the slides and fixed by using a vapor of ethanol, acetic acid, and distilled water in a ratio of 1:2:3. After being fixed for 2 hours, the slides were stained in a 2% Giemsa solution (pH 6.8-7.0), and photographed using a camera fixed on a Nikon microscope. For detailed information, refer to Tan et al. (1987a). Terminology was adopted from Levan et al. (1964).

Results

More than 50 metaphase chromosome spreads were observed and counted. *Rhacophorus reinwardtii* has a diploid chromosome number of 26. The karyotype

TABLE 1. Proportional data from 10 well spread metaphase chromosomes.

Chromosome no.	Relative Length	Arm Ratio	Centromeric Type
1	14.83 ± 0.63	1.30 ± 0.15	metacentric
2	12.14 ± 0.50	1.89 ± 0.22	submetacentric
3	11.14 ± 0.55	1.83 ± 0.22	submetacentric
4	10.69 ± 0.44	1.73 ± 0.19	submetacentric
5	09.61 ± 0.24	1.26 ± 0.07	metacentric
6	06.32 ± 0.28	1.39 ± 0.15	metacentric
7	05.89 ± 0.24	1.40 ± 0.17	metacentric
8	05.78 ± 0.20	1.27 ± 0.09	metacentric
9	05.40 ± 0.23	1.44 ± 0.21	metacentric
10	04.96 ± 0.31	1.23 ± 0.17	metacentric
11	04.61 ± 0.28	1.19 ± 0.13	metacentric
12	04.28 ± 0.30	1.15 ± 0.10	metacentric
13	04.02 ± 0.35	1.26 ± 0.15	metacentric

of *Rhacophorus reinwardtii* consists of 5 pairs of large (relative length larger than 9%) and 8 pairs of small (relative length smaller than 7%) chromosomes (Table 1).

Discussion

Rhacophorus reinwardtii has a karyotype of $2n=26$ with 5 large and 8 small pairs of chromosomes. This is often seen in the Rhacophoridae and may be either a primitive retention, or a synapomorphy. Many ranid frogs which are thought to be sister groups of the family Rhacophoridae (Inger 1967, Duellman 1988) have a similar karyotype.

As we have pointed out previously, the arm ratios of the chromosomes are useful in differentiating closely related species (Zhao et al. 1987). Moreover, Morescalchi (1973) proposed that there may be a relationship between how primitive the chromosomes are and the chromosomal arm ratios: the more primitive species tend to have more chromosomes with submetacentric, subtelocentric and telocentric centromeres. This hypothesis has been corroborated in most of the groups we have studied (Tan et al 1987a). This rather specialized and advanced



FIG. 1. Metaphase chromosomes of *Rhacophorus reinwardtii*. Arrows indicate the secondary constrictions.

treefrog (Jiang et al. 1987) with only 3 pairs of submetacentric chromosomes, supports this hypothesis. Exceptions to this generalization also have been observed in some groups (refer to Zhao et al. 1987). Attention should be paid to different members of closely related groups when

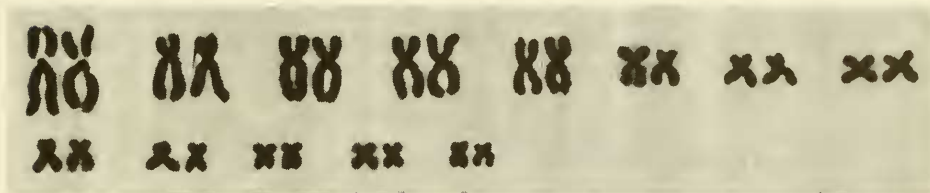


FIG. 2. Karyotype of metaphase chromosomes from figure 1.

studying chromosomal evolution and phylogeny.

The position of the secondary constriction on the chromosomes is another important criterion in cytotaxonomy. As shown in figures 1 and 2, *Rhacophorus reinwardtii* has a prominent secondary constriction in the paracentric regions of the short arms of the largest no. 1 chromosome. This is presumed to be the Nuclear Organizing Regions (NORs). In contrast, *Rhacophorus chenfui* (= *Polypedates chenfui*) has a prominent satellite on the terminal of the long arm of no. 11 chromosome, which has been shown to be the NORs.

To date several authors have reported on the classification of the Rhacophoridae which has a distribution limited to the Old World. Liem's family revision (1970) divided it into 14 genera in the Orient, Madagascar and the African tropics. Frost (1985) recognized 2 subfamilies (Philautinae and Rhacophorinae) and 10 genera. Jiang et al. (1987) studied 14 species of Chinese Rhacophoridae and identified 5 genera (*Buergeria*, *Philautus*, *Polypedates*, *Rhacophorus* and *Chirixalus*) instead of the 2 previously recognized genera (*Rhacophorus* and *Philautus*). The karyotypes of *Polypedates dennysi* (= *Rhacophorus dennysi*), *P. leucomystax* (= *Rhacophorus leucomystax*), *P. chenfui* (= *Rhacophorus chenfui*), *Chirixalus doriae* (= *Philautus doriae*) and *Rhacophorus reinwardtii* have been reported. Further studies should be carried out in order to give convincing evidence for the taxonomical position and phylogeny of the Chinese rhacophorid frogs.

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